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IDENTIFICATION OF SANDFLIES (Diptera: Psychodidae: Phlebotominae) BLOOD MEALS IN AN ENDEMIC LEISHMANIASIS AREA IN BRAZIL

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SUMMARY

The aim of this study was to identify blood meals of female sandflies captured in the municipality of Governador Valadares, an endemic area of visceral and cutaneous leishmaniasis, in the State of Minas Gerais, Brazil. From May 2011 to January 2012, captures were performed using HP light traps in four districts. There were 2,614 specimens (2,090 males and 524 females) captured; 97 engorged females were identified belonging to the species *Lutzomyia longipalpis* (82.1%) and *Lutzomyia cortelezzii* (17.9%). Considering simple and mixed feeding, the enzyme-linked immunosorbent assay revealed a predominance of chicken blood (43.6%) in *Lutzomyia longipalpis*, showing the important role that chickens exert around the residential areas of Governador Valadares. This finding increases the chances of sandflies contact with other vertebrates and consequently the risk of leishmaniasis transmission.

KEYWORDS: Sandflies; *Lu. cortelezzii*; *Lu. longipalpis*; Blood meal identification; ELISA.

INTRODUCTION

In the Americas, the transmission of *Leishmania* (Kinetoplastida, Trypanosomatidae) occurs mainly through the bite of female sandflies species of the genus *Lutzomyia* (Diptera: Psychodidae: Phlebotominae). These females need blood for the maturation of their ovarioles; they suck many vertebrates, including amphibians, reptiles, birds and mammals, the latter retaining reservoirs of *Leishmania*[10,16].

Sandfly species have the ability to adapt to different habitats. The diversity of species found next to human dwellings, with a plasticity in the face of changes brought about by humans, have contributed to the urbanization of leishmaniasis[3,23]. The identification of sandfly blood meal has been shown as an alternative to know how domestic and synanthropic animals can be effectively used as blood sources[6,18]. The identification of these blood sources can provide data on possible reservoirs of *Leishmania*, as well as the role that some animals might play in the transmission of parasites[7]. Many techniques have been used to identify blood sources for bloodsucking insects (i.e., precipitin test, gel electrophoresis, PCR and ELISA), highlighting the most commonly used, the immunoenzymatic techniques[12,14,20,24].

In Brazil, *Lutzomyia longipalpis* (Lutz & Neiva 1912) is a frequent subject of studies on blood meal[1,15], probably because of its importance as a main vector of *Leishmania infantum* in different geographical regions[13]. In contrast, the literature on the feeding behavior of other non-vector species is quite rare. ROSA *et al*. (2012)[22] raised the possibility of *Lutzomyia cortelezzii* complex be participating in the transmission of *Leishmania* in areas of cutaneous leishmaniasis because they have been found naturally infected and in high abundance in these areas. In Brazil, CARVALHO *et al*. (2008)[8] found this species naturally infected by *Le. infantum* (syn *Leishmania chagasi*) in the Santa Luzia municipality, in the State of Minas Gerais.

The aim of this study was to identify the blood meals of females from the species *Lu. longipalpis* and *Lu. cortelezzii* in an area of simultaneous occurrence of visceral and cutaneous leishmaniasis, to contribute to the knowledge of the biology and behavior of these species and their roles in the epidemiology of leishmaniasis.

MATERIAL AND METHODS

STUDY AREA: In the municipality of Governador Valadares (18°51'12" S - 41°56'42" W), Minas Gerais State, Brazil (Fig. 1), from May 2011 to January 2012, phlebotomine captures were performed using 16 HP light traps[19] exposed inside and outside the homes of eight fixed residences in four neighborhoods in the urban area with reported human cases of visceral (VL) and cutaneous leishmaniasis (ACL) (Altinopolis, Mae de Deus, Nossa Senhora das Graças and Santa Helena). In general, the residences had courtyards, with the continuous presence of domestic

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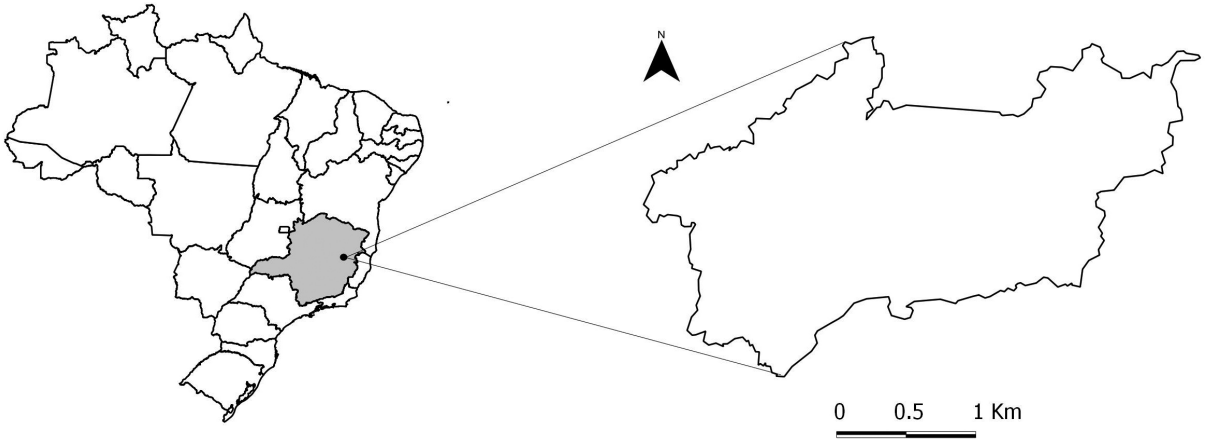

Fig. 1 - Geographical localization of the Municipality of Governador Valadares, Minas Gerais State, Brazil.

animals such as dogs, chickens, and cats. The presence of rodents was mentioned by residents. Sandflies were captured with traps assembled at 4:00 P.M. and removed the following day at 8:00 A.M., for three consecutive nights each month.

Enzyme-linked immunosorbent assay: After the capture, the specimens were killed by freezing them at -20 °C for complete paralysis of the digestive process. Then, the specimens' head and last two segments of the abdomen were removed to identify the species of sandfly according to the classification proposed by YOUNG & DUNCAN (1994)[25]. The identification of blood meal was performed according to the enzyme-linked immunosorbent assay proposed by BURKOT *et al*. (1981)[7] modified by DUARTE (1997)[11], using four antisera: chicken, dog, rodent and human, for each specimen (see, MARASSÁ *et al*. 2004)[14].

RESULTS

In the municipality of Governador Valadares, 2,614 specimens were captured (2,090 males and 524 females) (see, BARATA *et al*. 2013) of which 97 were engorged. The distribution of phlebotomine females per district and environment is shown in Table 1. Engorged females were identified as belonging to the species *Lu. longipalpis* (82.1%) and *Lu. cortelezzii* (17.9%). The identification of blood-feeding females according to the ELISA test can be viewed in Table 2. The record of these species occurred predominantly in the peridomicile (69.4%), but also inside homes (30.6%). Two other species were captured in this study: *Lutzomyia intermedia* (Lutz & Neiva 1912) and *Lutzomyia whitmani* (Antunes & Coutinho, 1939) (data not shown)[5], but they were not engorged. Simple feeding was observed in 79.6% of females analyzed while 20.4% fed on more than one source of blood (Table 2).

DISCUSSION

Considering simple and mixed feeding, we observed more positive results to chicken antiserum in *Lu. longipalpis*, but also found sandflies engorged with the blood of dogs, rodents and humans. *Lutzomyia cortelezzii* seems to follow the same eclectic feeding behavior and preference of *Lu. longipalpis*. The presence of more than one source of blood in some females reinforces the food eclecticism of these species, demonstrating that sandflies usually adjust their feeding pattern according to the availability of hosts[17].

Attraction of *Lu. longipalpis* to chickens has already been reported in the literature[2,4]. It is evident that this association contributes to the domiciliation of *Lu. longipalpis*. Chickens attract insects in the peridomicile area increasing the chances of sandflies contact with other vertebrates and thus increasing the risk of leishmaniasis transmission. This finding can play an important epidemiological role, contributing to the maintenance of breeding places for sandflies.

Dogs play an important role in the maintenance of visceral leishmaniasis in the human environment, serving as reservoirs for this intracellular parasite[21]. Analyzing Table 2, dogs also appeared as alternative sources of blood meal. Thus, the presence of sandflies inside

Table 1
Distribution of phlebotomine females collected per district and environment, in the municipality of Governador Valadares, state of Minas Gerais, between May 2011 and January 2012

Species	District								Total
	Altinópolis		Mae de Deus		N. Sra das Graças		Santa Helena		
	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	
Lu. cortelezzii	1	2	2	-	-	3	-	3	11
Lu. longipalpis	11	3	6	35	4	24	1	2	86
Total	12	5	8	35	4	27	1	5	**97**

Table 2
Percentage of engorged female sandflies females analyzed by ELISA**,** collected in the municipality of Governador Valadares, state of Minas Gerais, between May 2011 and January 2012

Feeding	Antisera	*Lu. cortelezzii*		*Lu. longipalpis*		N	Total (%)
		Inside (%)	Outside (%)	Inside (%)	Outside (%)		
Simple	chicken	5.2	-	5.2	28.2	37	38.6
	dog	-	7.7	12.7	5.2	25	25.6
	human	-	-	-	5.2	5	5.2
	rodent	-	2.5	2.5	5.2	10	10.2
	Subtotal	**5.2**	**10.2**	**20.4**	**43.8**	77	**79.6**
Mixed	chicken + dog	-	-	-	2.5	2	2.5
	chicken + human	-	-	2.5	-	2	2.5
	chicken + rodent	-	2.5	-	5.2	8	7.7
	dog + rodent	-	-	2.5	5.2	8	7.7
	Subtotal	**0**	**2.5**	**5.0**	**12.9**	20	**20.4**
Total		**17.9**		**82.1**		97	**100**

N = absolute number of sand flies engorded.

and outside homes, as mentioned earlier, can increase the contact of sandflies with humans, maximizing the probability of infection by *Leishmania*.

Human blood was detected only in *Lu. longipalpis*. The observation that *Lu. longipalpis* females fed on humans demonstrates that this species exhibits an anthropophilic behavior. In addition, the ability to feed on a variety of host species and also mixed feeding profiles (chicken+dog/ chicken+human/ chicken+rodent/ dog+rodent) reinforces the role of sandflies as the main vectors of VL in Brazil.

Finally, rodents were also in the group of animals used in sandflies blood meals. This finding reinforces the need to study rodents as possible reservoirs of *Leishmania* sp., as suggested by CORREDOR *et al*. (1989)[9], because they constitute an important link between the forest and domiciliary environments.

RESUMO

Identificação do repasto sanguíneo de flebotomíneos (Diptera: Psychodidae: Phlebotominae) provenientes de área endêmica de leishmaniose no Brasil

O objetivo deste estudo foi identificar o repasto sanguíneo de fêmeas de flebotomíneos capturadas no município de Governador Valadares, área endêmica de leishmaniose visceral e tegumentar no Estado de Minas Gerais, Brasil. Entre maio de 2011 e janeiro 2012 foram realizadas capturas com armadilhas luminosas HP em quatro bairros. Foram capturados 2.614 exemplares (2.090 machos e 524 fêmeas). Noventa e sete fêmeas ingurgitadas foram identificadas como pertencentes às espécies *Lutzomyia longipalpis* (82,1%) e *Lutzomyia cortelezzii* (17,9%). Considerando a alimentação simples e a mista, o ensaio imunoenzimático revelou em *Lutzomyia longipalpis* uma predominância de sangue de galinhas (43,6%), mostrando o importante papel que galinhas podem exercer no peridomicílio, aumentando a chance de contato dos flebotomíneos com outros vertebrados e, consequentemente, o risco de transmissão da leishmaniose.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Afonso MM, Duarte R, Miranda JC, Caranha L, Rangel EF. Studies on the feeding habits of *Lutzomyia (Lutzomyia) longipalpis* (Lutz & Neiva 1912) (Diptera: Psychodidae: Phlebotominae) populations from endemic areas of American visceral leishmaniasis in Northeastern Brazil. J Trop Med. 2012;2012:858657

2. Alexander B, de Carvalho RL, McCallum H, Pereira MH. Role of the domestic chicken (*Gallus gallus*) in the epidemiology of urban visceral leishmaniasis in Brazil. Emerg Infect Dis. 2002;8:1480-5.

3. Badaró R, Jones TC, Lorenço R, Cerf BJ, Sampaio D, Carvalho EM, *et al.* A prospective study of visceral leishmaniasis in an endemic area of Brazil. J Infect Dis. 1986;154:639-49.

4. Barata RA, França-Silva JC, Mayrink W, Silva JC, Prata A, Lorosa ES*, et al.* Aspectos da ecologia e do comportamento de flebotomíneos em área endêmica de leishmaniose visceral, Minas Gerais. Rev Soc Bras Med Trop. 2005;38:421-5.

5. Barata RA, Peixoto JC, Tanure A, Gomes ME, Apolinário EC, Bodevan EC, *et al.* Epidemiology of visceral leishmaniasis in a reemerging focus of intense transmission in Minas Gerais State, Brazil. Biomed Res Int. 2013;2013:405083

6. Boreham PF. Some applications of bloodmeal identifications in relation to the epidemiology of vector-borne tropical diseases. J Trop Med Hyg. 1975;78:83-91.

7. Burkot TR, Goodman WG, DeFoliart GR. Identification of mosquito blood meals by enzyme-linked immunosorbent assay. Am J Trop Med Hyg. 1981;30:1336-41.

8. Carvalho GM, Andrade Filho JD, Falcão AL, Rocha Lima AC, Gontijo CM. Naturally infected *Lutzomyia* sand flies in a *Leishmania*-endemic area of Brazil. Vector Borne Zoonotic Dis. 2008;8:407-14.

9. Corredor A, Gallego JF, Tesh RB, Peláez D, Diaz A, Montilla M, Paláu MT. *Didelphis marsupialis*, an apparent wild reservoir of *Leishmania donovani chagasi* in Colombia, South America. Trans R Soc Trop Med Hyg. 1989;83:195.

10. Deane LM, Deane MP. Leishmaniose visceral urbana (no cão e no homem) em Sobral, Ceará. O Hospital. 1955;47:75-87.

11. Duarte R. Ensaio imunoenzimático ELISA para identificação experimental de fontes alimentares em *Panstrongylus megistus* (Burmeister, 1835) (Hemiptera: Reduviidae). [Dissertação]. Rio de Janeiro: Instituto Oswaldo Cruz/Fundação Oswaldo Cruz; 1997.

12. Ferreira FS. A reação das precipitinas aplicadas aos dípteros do gênero *Phlebotomus*. An Inst Med Trop. 1945;2:187-96.

13. Lainson R, Rangel EF. *Lutzomyia longipalpis* and the eco-epidemiology of American visceral leishmaniasis, with particular reference to Brazil: a review. Mem Inst Oswaldo Cruz. 2005;100:811-27.

14. Marassá AM, Consales CA, Galati EA. Padronização da técnica imunoenzimática do ELISA de captura, no sistema avidina-biotina para a identificação de sangue ingerido por *Lutzomyia (Lutzomyia) longipalpis* (Lutz & Neiva, 1912). Rev Soc Bras Med Trop. 2004;37:441-6.

15. Missawa NA, Lorosa ES, Dias ES. Preferência alimentar de *Lutzomyia longipalpis* (Lutz & Neiva, 1912) em área de transmissão de leishmaniose visceral em Mato Grosso. Rev Soc Bras Med Trop. 2008;41:365-8.

16. Morrison AC, Ferro C, Tesh RB. Host preferences of the sand fly *Lutzomyia longipalpis* at an endemic focus of American visceral leishmaniasis in Colombia. Am J Trop Med Hyg. 1993;49:68-75.

17. Muniz LH, Rossi RM, Neitzke HC, Monteiro WM, Teodoro U. Estudo dos hábitos alimentares de flebotomíneos em área rural no sul do Brasil. Rev Saúde Pública. 2006;40:1087-93.

18. Ngumbi PM, Lawyer PG, Johnson RN, Kiilu G, Asiago C. Identification of phlebotomine sandfly bloodmeals from Baringo District, Kenya, by direct enzyme-linked immunosorbent assay (ELISA). Med Vet Entomol. 1992;6:385-8.

19. Pugedo H, Barata RA, França-Silva JC, Silva JC, Dias ES. HP: um modelo aprimorado de armadilha luminosa de sucção para a captura de pequenos insetos. Rev Soc Bras Med Trop. 2005;38:70-2.

20. Quaresma PF, Carvalho GM, Ramos MC, Andrade-Filho JD. Natural *Leishmania* sp. reservoirs and phlebotomine sandfly food source identification in Ibitipoca State Park, Minas Gerais, Brazil. Mem Inst Oswaldo Cruz. 2012;107:480-5.

21. Quinnell RJ, Dye C, Shaw JJ. Host preferences of the phlebotomine sandfly *Lutzomyia longipalpis* in Amazonian Brazil. Med Vet Entomol. 1992;6:195-200.

22. Rosa J, Pereira DP, Brazil RP, Filho JD, Salomón O, Szelag E. Natural infection of cortelezzii complex (Diptera: Psychodidae: Phlebotominae) with *Leishmania braziliensis* in Chaco, Argentina. Acta Trop. 2012;123:128-31.

23. Silva ES, Gontijo CM, Pacheco RS, Fiuza VO, Brazil RP. Visceral leishmaniasis in the Metropolitan Region of Belo Horizonte, state of Minas Gerais, Brazil. Mem Inst Oswaldo Cruz. 2001;96:285-91.

24. Srinivasan R, Panicker KN. Identification of bloodmeals of phlebotomine sandflies using the agarose gel diffusion method. Southeast Asian J Trop Med Publ Health. 1992;23:486-8.

25. Young DG, Duncan MA. Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). Mem Am Entomol Inst. 1994;54.

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